



## **The Effect of Thidiazuron on Callus and *in vitro* Shoots Development of Date Palm (*Phoenix dactylifera* L.) cv. Barhee**

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**Abstract:** Date palm (*Phoenix dactylifera* L.) is one of the most important fruit trees spread in southern Iraq, but because of many circumstances decreased their numbers significantly, the propagation of date palm by tissue culture was the best solution for re-preparation of the problem as it was previously, because propagation is accelerated. Summarizes the working paper in an attempt to find the optimal concentration of thidiazuron (TDZ) (0.0 , 0.5 and 1.0) mg l<sup>-1</sup>, in production of callus and regeneration of shoots, at different times of initial and callus culturing in medium budding. The results showed that the concentration of (0.5) mg l<sup>-1</sup> was optimal in all traits under study.

**Key words:** *Phoenix dactylifera*, Thidiazuron (TDZ), Callus, Shoot multiplication, browning, *in vitro*.

### **Introduction**

Date palm (*Phoenix dactylifera* L) is one of the most important fruit trees evergreens in many countries of the world, the originate of the date palm is believed in the Arabian Gulf and it is likely to have originated in southern Iraq (Wrigley, 1995).

Traditional method of date palm multiplication is achieved by offshoots. The limited availability of offshoots makes this traditional propagation method insufficient, especially since some cultivars do produce a few offshoots for a certain period in the life time of a young palm tree, slow propagation rate, the offshoots are also a source of transportation of diseases and insects (Gueye *et al.*, 2009). Tissue culture techniques have

become a common and alternative method for vegetative propagation of date palm in recent years. Especially as date palm responds to modern biotechnology applications (Jasim *et al.*, 2009). The benefit of using this technique is the production of true to type, disease free. Moreover production is on a commercial scale (Al-Wasel, 2001; Ahloowalia *et al.*, 2004). Thiaduzuron (TDZ) (N-phenyl-N- 1, 2, 3-thiadiazol-5-ylurea). Cappelletti *et al.* (2016) displayed that the addition of both 0.2 mg l<sup>-1</sup> and 0.5 mg l<sup>-1</sup> of TDZ in the medium led to improved callus formation in blueberry. The formation of shoot is stimulated in a wide range of plant species chestnut, mulberry, white pine, banana Pinus and strawberry (San-Jose *et al.*, 2001; Chitra & Padmaja 2005; Tang & Newton, 2005; Gubbuk and

Pekmezci, 2006; Jericó *et al.*, 2011; Cappelletti *et al.*, 2016). As for studies on date palms, Al-Mayahi (2014) explained that the transfer of callus to the multiplication medium supplemented with 0.5 mg.l<sup>-1</sup> TDZ, recorded the best response (66.67%), with the highest rate of number of shoots 18.2 shoot after 24 weeks from culturing. Although the progress made in the cultivation of plant tissues, there are many problems and difficulties in propagation of date palm in this way. The composition of the growth media is of great importance in the growth and development of cultured plants. This reflects the success of propagation of this technique. A statistics of date palm indicate a decrease in the numbers of this tree in all areas of palm growing in Iraq, especially in the province of Basrah because of the conditions that have led to the neglect the farmers to orchards, as well as the scarcity of irrigation water and increase salinity. The aim of this study is focusing on the effect of thiaduzuron ( TDZ) on the growth and development of callus and regenerated buds of date palm Barhee cultivar

## Materials & Methods

The experiments for this study were carried out in tissue culture laboratory for Date Palm Research centre in University of Basrah.

### 1-Effect of different concentrations of Thiaduzuron (TDZ) on growth primary callus at different times of callus culturing

Callus of date palm cv. Barhee obtained from the cultivating the quarters of apical buds on medium composed of (MS) (Murashig & Skoog, 1962), with additional 30 mg l<sup>-1</sup>  $\alpha$ -naphthalene acetic acid (NAA), 3mg l<sup>-1</sup> isopentenyl adenine (2iP) and 2 g l<sup>-1</sup> activated charcoal, under complete darkness at 27  $\pm$  2 °C. The callus was divided and re-culture into jars contain 100 mg MS media with the

addition of the following chemicals (mg.l<sup>-1</sup>) as sodium phosphate (170), thiamine (0.5), adenine sulfate (40), (NAA) 10 and cytokinin (2iP) 1.5 mg l<sup>-1</sup>. TDZ has been added with different concentrations (0.0, 0.5 and 1.0) mg.l<sup>-1</sup>, to examine its effect on the growth of initial callus after four, six and eight weeks, which was adopted as an indicator of growth according to the method mentioned by (Jasim & Saad, 2011).

### 2-Test of Thiaduzuron (TDZ) on stimulation of primary callus tissue to budding

A 100 mg of callus were transferred to a jar that contains MS media with the additional growth regulators 1mg.l<sup>-1</sup> NAA and 3 mg.l<sup>-1</sup> 6-benzylamino purine (BA) and activated charcoal at 500 mg. l<sup>-1</sup>. TDZ was added at concentrations of 0.0, 0.5 and 1.0 mg.l<sup>-1</sup>. Six replicates were used for each treatment. The cultures were incubated at a temperature of 27  $\pm$  1 ° C, a light intensity of 1000 lux with a rate of 16 light hours/day followed by 8 hours of darkness, The following measurements were taken after 10,12 and 14 weeks of callus culturing. i- Percentage of callus tissue budding, ii- percentage of browning.

### 3-Test The thiaduzuron (TDZ) on the growth and multiplication of adventitious buds

Several concentrations of Thiaduzuron (TDZ) (0.0, 0.5 and 1.0) mg.l<sup>-1</sup> were tested in order to investigate their effect on the growth of adventitious buds and their multiplication of date palm cv. Barhee grown *in vitro*, The buds were divided from two - three buds and re-culture in MS media with addition 0.5 mg l<sup>-1</sup> NAA 0.5 mg l<sup>-1</sup>BA, 0.5 mg l<sup>-1</sup> kinetin (K) and 0.5 g l<sup>-1</sup>activated charcoal 0.5 mg l<sup>-1</sup>(Al-Mayahi, 2016). The measurements were taken after 4,6 and 8 weeks of culturing, included

1) Number of buds. 2) Number of shoots. 3) Weight of buds.

### Experimental design and statistical analysis

The experiment was design in Completely Randomized Design (C.R.D). Using GenStat software. Differences were compared using the least significant difference (L.S.D) at the 5% probability level, depending on (Snedecor & Cochran, 1989).

### Result & Dissection

The results in fig. (1) shown significant effect of Thiaduzuron on the weight of callus and It is clear that increase the weight of callus by increasing the duration of callus culture and the concentration of TDZ, The weight after

four weeks was increased in the treatment of 1 mg<sup>l</sup><sup>-1</sup> TDZ. Which gave a higher weight reached 1.58 g which no significant difference with the treatment 0.5 mg.l<sup>-1</sup>, while a significant difference with control treatment. After six weeks of callus culture, there was a significant increase in the weight of the callus, which reached 5.94 g and 5.63 g for both treatments 0.5 and 1 mg l<sup>-1</sup> respectively, which indicated a significant difference compared to control treatment 3.12 g. after 8 weeks of culture, the weight of the callus increasing reached the highest weight reached 5.63 g and 5.49 g for the treatments 0.5 and 1 mg l<sup>-1</sup> respectively, which revealed a significant difference compared to control treatment.

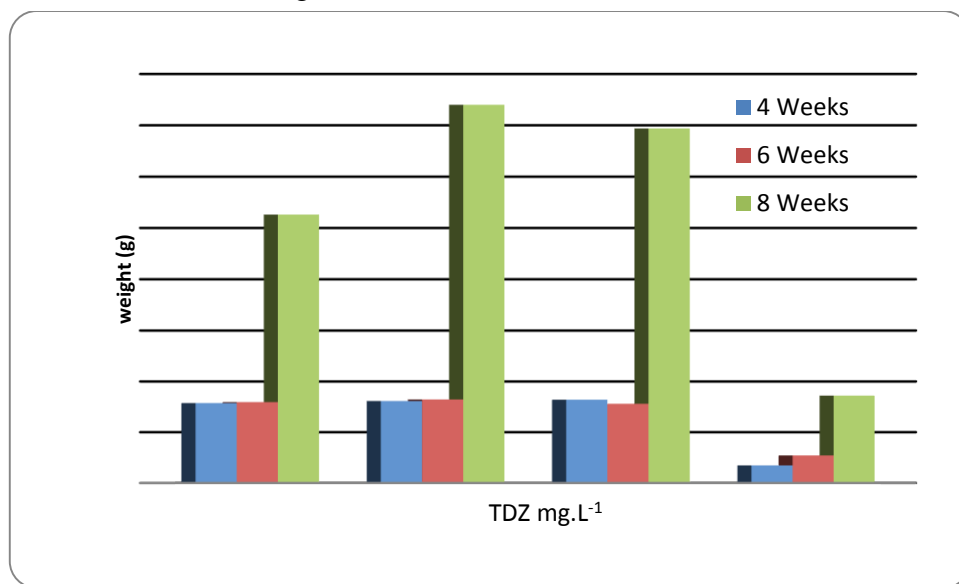


Fig. (1): Effect of TDZ on callus fresh weight.

Fig. (2) exhibited the effect TDZ concentrations on callus browning percentage. It is clear from the figure that the highest percentage obtained in the control treatment was 8.3% followed by the treatment of 1 mg l<sup>-1</sup>, while the treatment of 0.5 mg l<sup>-1</sup>, gave a lower percentage of browning reach 1.9%.

Fig. (3) presented the percentage of budding of callus tissue, that the highest percentage of callus budding at treatment 0.5 mg l<sup>-1</sup> was reached 40% and significantly different with two other treatments 1 mg l<sup>-1</sup> and control treatment 33 and 28%, respectively.

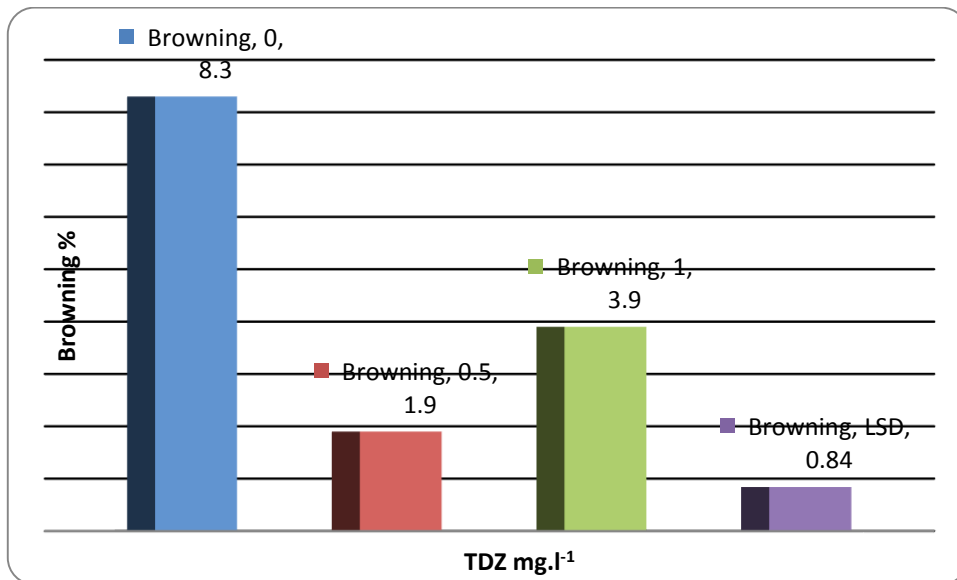


Fig. (2): Effect of TDZ on callus browning percentage.

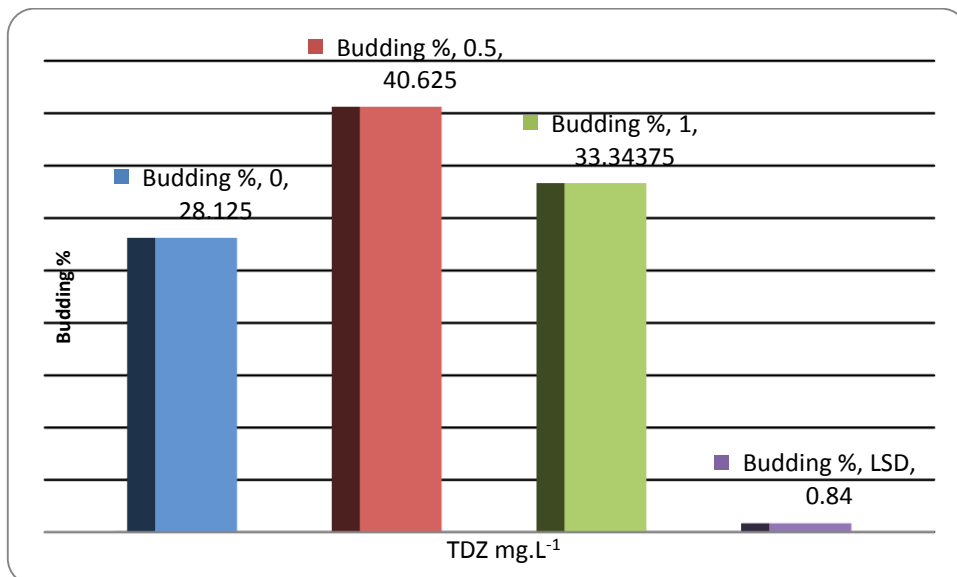
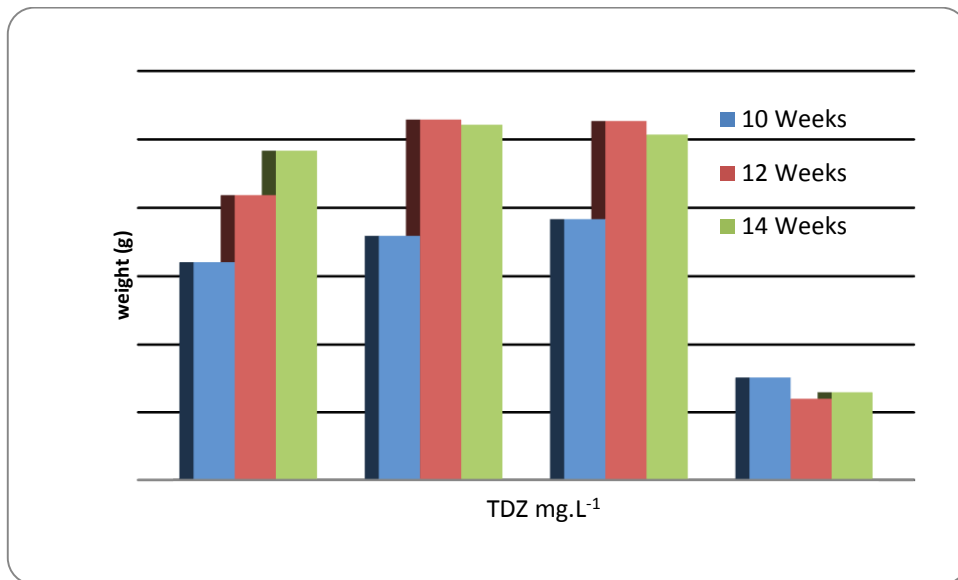


Fig. (3): Effect of TDZ on a response percentage of callus on budding.

The effect of Thiaduzuron on the weight of buds during the culturing period. There was no significant difference after 10 weeks of cultivation in the weight of buds. The highest weight recorded by the concentration of 1 mg.l<sup>-1</sup> was 7.76 g then 7.18g in 0.5mg.l<sup>-1</sup> treatment. Buds weight were increased after 12 weeks of culturing, the two treatment 0.5 and 1 mg l<sup>-1</sup> recorded 10.58 g and 10.54 g

respectively, which displayed a significant difference compared to control treatment. Thereafter, a slight decrease in weight it has notes at 14 weeks after cultured, treatment of 0.5 mg l<sup>-1</sup> recorded a weight reached 10.43 g with no significant difference with control treatment. The decrease of buds weight may be due to an increase in the number of buds on the area unit which have led to competition

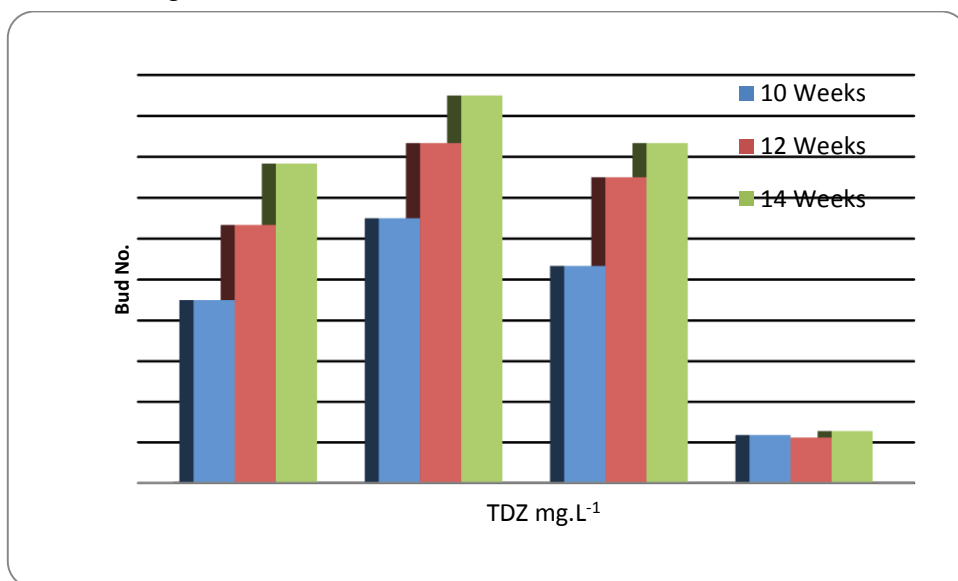


**Fig. (4): Effect of TDZ on buds weight.**

for available food resources, thus causing a decline in their growth as illustrated by Fig. (4). Fig. (5) indicated significant differences in the number of shoots after 10 weeks were gave 13 buds at 0.5 mg l<sup>-1</sup> which showed significant difference from 1 mg l<sup>-1</sup> concentration 10.67 buds which did not differ significantly from the control treatment, which recorded 9 buds, and then noted a linear increase with the increased time with the highest number of buds at treatment 0.5 mg l<sup>-1</sup>, treatment 1 mg l<sup>-1</sup> after 12 weeks

reached 16.67 and 15, respectively, The highest number of shoots after 14 weeks of culturing was 19 bud at 0.5 mg l<sup>-1</sup> and a significant difference from the treatment 1 mg l<sup>-1</sup>, which in turn did not differ significantly from the control treatment.

The effect of Thidiazuron on shoots number was no significant difference in shoots number during the first and second dates (10 and 12 weeks).



**Fig. (5): Effect of TDZ on Buds Number.**

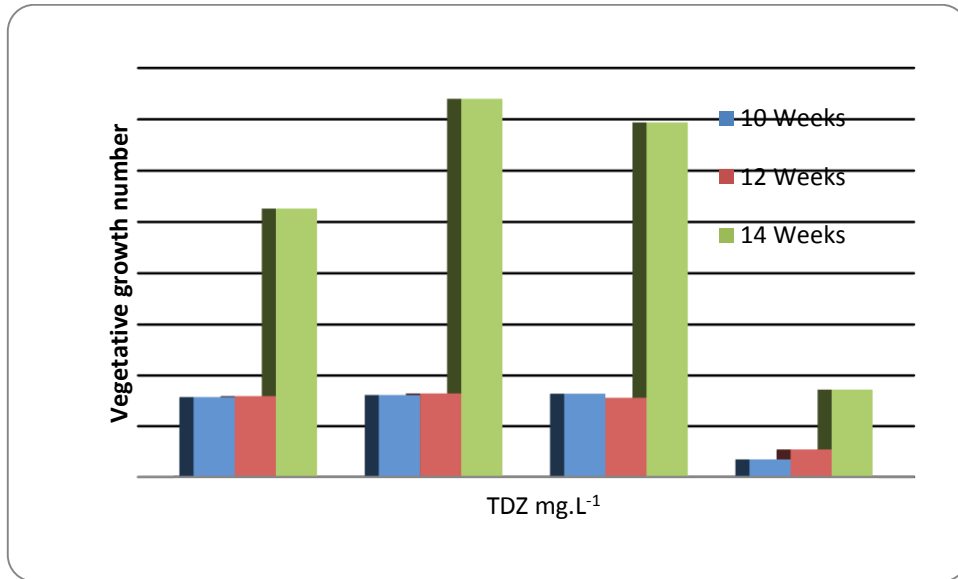


Fig. (6): Effect of Thidiazuron vegetative growth number.

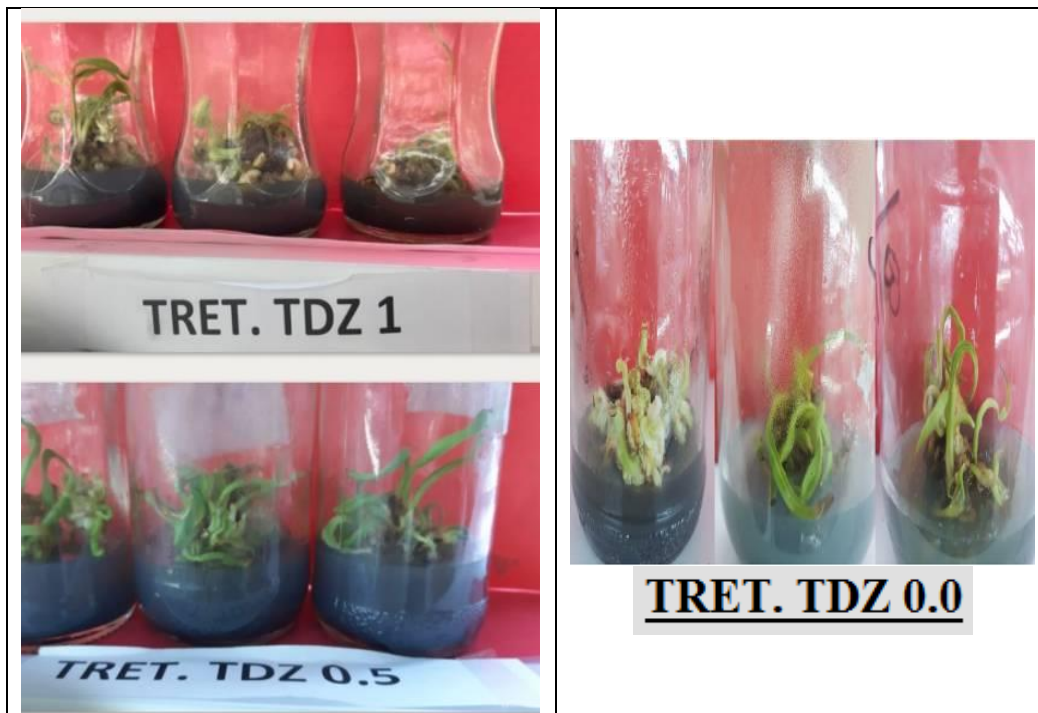


Fig. (7): Multiple shoot proliferation of date palm cv.Barhee *in vitro* under different concentration of TDZ.

As well as, statistical analyzes did not show any significant differences between different concentrations on the number of shoots. However, the highest regeneration in shoot

number was recorded on the third date after 14 weeks of re-culture. The treatment was 0.5 mg l<sup>-1</sup> gave a high number of shoot growths

which reached 37 shoot growth followed by the treatments 1 and 0 mg l<sup>-1</sup> (Figs. 6 & 7).

The effects of Thiaduzuron on callus fresh weight is a result of its effectiveness in stimulating the division of cells and then increase the weight by stimulating the building of proteins, enzymes and the RNA molecule inside the cell, which is reflected on the increase in cells size, as a result of the transfer of water and food to them. Thiadozorone works to balance or modify the internal levels of auxins and cytokinins within cells, which it is reflected on the increase the number of lateral buds and their weight as a result of the increase of lateral growth, thiadiazurone also reduces the disruption of chlorophyll and protein, and stimulation the enzymes of photosynthesis, thus increasing the number of buds and shoots length (Abbasi *et al.*, 2011; Thomas, 2003; Casanova *et al.*, 2004; Al-Mayahi, 2014).

## Conclusion

It seems from this paper the advantage of using Thiaduzuron and consideration as a suitable hormone at 0.5 mg.l<sup>-1</sup> to give a high weight of callus of Barhee date palm cultivar and increased the number of buds and increase the shoot development.

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